iGEM Bordeaux 2015

Lab Notebook of June

Abbreviations:
QS: Quantum sufficit = sufficient quantity for

02/06/2015

Goal :
Made some medium and stock solution to prepare protocols for Competent cells.

Procedure :

- $MgSO_4$ Solution Stock : 0,5M

For 500mL :
- 61,62g of $MgSO_4$ (MM : 246,48)
- QS 500mL H_2O

- SOB Medium : 1 L

Final [c]	Component	Volume & Mass
2 %	Bactotryptone	20 g
0,5 %	Yeast Extract	5 g
10 mM	NaCl	2 ml (5 M stock)
2,5 mM	KCl	0,18 g
10 mM	$MgCl_2$	5 ml (2 M stock)
10 mM	MgSO4	20 mL (0,5 M)
-	H_20	qsp 1L

adjust pH media to 7 with NaOH, then autoclave.

- 2XTY Medium

Component	Volume & Mass
Bactotryptone	8 g
Yeast Extract	5 g
NaCl	2,5 g
H_20	QS 500 mL

03/06/2015

Goal :
Made medium to prepare the Competent cells protocol and started the pre-culture of futur competent cells.

Procedure :

- Transformation Broth (TB): 1 L

Final [c]	Component	Volume & Mass
10 mM	Pipes	3.02 g
15 mM	$CaCl_2$	2.21 g
250 mM	KCl	18.64 g

Mix the Pipes, $CaCl_2$, and KCl in 900 ml of millipore water. Add NaOH until pH is 6.7, **Don't worry, dust disappear after pH adjust.**
Add MnCl2 (see below), stir, adjust volume to 1 L, then filter sterilize. Store at 4C.

Final [c]	Component	Volume & Mass
55 mM	$MnCl_2$	10.89 g

- Making DMSO Competent Cells

DAY ONE: Grow 12 ml overnight culture of favorite strain of E. coli in 2XTY at 37°C and 180 rpm (We use Denis' DH5-alpha).

04/06/2015

Goal :
Made antibiotic stock solution and continued the protocol for competent cells.

Procedure :

- Making DMSO Competent Cells

DAY TWO:
Inoculate 1 L SOB with 12 ml overnight culture.
Keep 5mL of SOB for initial OD.
Grow culture at 18C and 180 rpm (this temperature is really important as we see a 10-fold decrease in competency when we grow them at room temperature).

- Chloramphenicol stock solution 34 mg.mL-1 (29,5 mL) :

Weigh out 1 g chloramphenicol and dissolve in 25 ml of 100% ethanol.
Make up volume to 29.5 ml with 100% ethanol.

<STORAGE> Aliquots of appropriate volume can be stored at -20 °C
10 µg/ml

- Tetracycline stock solution 12,5 mg.mL-1 (80 mL) :

Weigh out 1 g of tetracycline and dissolve in 75 ml of 1:1 vol/vol distilled water:ethanol.
Make up volume to 80 ml with 1:1 vol/vol distilled water:ethanol. (40mL ethanol + 40mL dH_2O)
Sterilize by filtration.

<STORAGE> Aliquots of appropriate volume should be wrapped in aluminium foil and stored at -20 °C.
12,5 µg/ml

05/06/2015

Goal :
Made some medium for futur bacterial transformation and continued the protocol for competent cells.

Procedure :

- **Medium preparation for bacterial transformation :**

LB agar (X3)	**250 mL**
Bactotryptone	2,5 g
Yeast Extract	1,25 g
NaCl	2,5 g
Agar	3 g
Adjust pH to 7.5 with NaOH and autoclave for 20 minutes	
+ Chloramphenicol (34 mg/ml in ethanol)	75 μL / 250 mL medium {10 μg/ml (final)}
+Tetracycline (12,5 mg/ml in 50% ethanol)	250 µL / 250 mL medium {12,5 µg/ml (final)}

LB liquid	**500 mL**
Bactotryptone	5 g
Yeast Extract	2,5 g
NaCl	5 g
Agar	6 g
Adjust pH to 7.5 with NaOH and autoclave for 20 minutes	

- Making DMSO Competent Cells

DAY THREE:
Grow cells until A600 0.5-0.7
Blank = SOB
A600 nm = 0,011
A600 nm = 0,008

Results : So we incubate during the week-end at 18°C and 120 rpm, and we hope that Monday OD will be good…

08/06/2015

Goal :
Made some medium for curdlan production and LB stock and continued the protocol for competent cells.

Procedure :

- Making DMSO Competent Cells

DAY THREE (Bis) :
Grow cells until A600 0.5-0.7
Blank = SOB
A600 nm = 0,008

Results : So we take an other protocol (open_wet_ware)

- **LB Liquid (3.5L)**

LB liquid	**1 L**
Bactotryptone	10 g
Yeast Extract	5 g
NaCl	10 g
Adjust pH to 7.5 with NaOH and autoclave for 20 minutes	

- **Preparation of 5X stock of M63 Medium (1L)**

Component	**Volume & Mass**	**Procedure**
$(NH_4)_2SO_4$	10 g	1) Add the following reagents to a 2-liter flask 2) Adjust volume to 1 L 3) Once the ingredients are added, heat with stirring until the components are completely dissolved. 4) Adjust pH to 7.0 with Acid 5) Sterilize by autoclave
KH_2PO_4	68 g	
$FeSO_4.7H_2O$	2,5 **mg**	

Problems : we have $FeSO_4$ precipitate + pH superior to 7
Hypothesis : we put 2,5g of $FeSO_4.7H_2O$ instead of 2,5mg

- Preparation of other Chemical Competent cells (open_wet_ware procedure)

TSS buffer (For 50 mL)
- 5g PEG 8000
- 1.5 mL 1M MgCl2 (or 0.30g $MgCl_2$-$6H_20$)
- Add LB to 50 mL
- Filter sterilize (0.22 µm filter)
- Add after sterilization 2.5 mL DMSO (the 09/06)

Overnight culture :
Grow a 5mL overnight culture of DH5-alpha in LB media at 37°C and 180 rpm.

09/06/2015

Goal :
Made Chemical competent cells with a new protocol.

Procedure :

- Preparation of other « Making Chemical Competent cells v2 »

1. In the morning, dilute this culture back into 50mL of fresh LB media in a 200mL conical flask, at 37°C and 180 rpm. (Dilute the overnight culture by at least 1/100).

Grow the diluted culture to an OD600 of 0.2 - 0.5.
We obtained : OD_{600nm} = 2,744
SO to obtain a good quantity of cells. We « diluted » the culture later

2. Put eppendorf tubes on ice **now**.

- Theoretically, if your culture is 50 ml, you will need 50 tubes. But **we had 5X more cells.** We will need **250 tubes.**

- At this point you should also make sure that your TSS is being chilled (it stored at 4°C).

3. Split the culture into two 50mL falcon tubes and incubate on ice for 10 min.
   TARE : tube 1 = 37,03g
   tube 2 = 37,00g

Remarks : All subsequent steps should be carried out at 4°C and the cells should be kept on ice wherever possible

4. Centrifuge for 10 minutes at 3000 rpm and 4°C.

5. Remove supernatant. The cell pellets should be sufficiently solid that you can just pour off the supernatant if you are careful. Pipette out any remaining media.

6. Resuspend in chilled TSS buffer.
The volume of TSS to use is 10% of the culture volume that you spun down => so **25 mL**
You may need to vortex gently to fully resuspend the culture. Keep an eye out for small cell aggregates even after the pellet is completely off the wall. We resuspended with pipetteman with a **cone cut at its end.**

7. Add 100 µl aliquots to your chilled eppendorfs and store at − 80°C.
One part was stored **directly at -80°C** and the other part was frozen **in liquid nitrogen.**

10/06/2015

Goal :

Test transformation efficiency of our competent cells v2.

Procedure :

- **Transformation Efficiency Kit iGEM**

1. Spin down the DNA tubes from the Transformation Efficiency Kit to collect all of the DNA into the bottom of each tube prior to use. A quick spin of 20-30 seconds at 8,000-10,000 rpm will be sufficient. Note: There should be 50 µL of DNA in each tube sent in the Kit.

2. Thaw competent cells on ice. Label one 2.0mL microcentrifuge tube for each concentration and then pre-chill by placing the tubes on ice.

3. Pipet 1 µL of DNA into each microcentrifuge tube. For each concentration, use a separate tube.

4. Pipet 50 µL of competent cells into each tube. Flick the tube gently with your finger to mix. Incubate on ice for 30 minutes. Pre-heat waterbath now to 42°C.

5. Heat-shock the cells by placing into the waterbath for 1 minute. Be careful to keep the lids of the tubes above the water level, and keep the ice close by.

6. Immediately transfer the tubes back to ice, and incubate on ice for 5 minutes. This helps the cells recover.

7. Add 200 µL of LB media per tube, and incubate at 37°C for 2 hours.

8. Prepare the agar plates during this time: label them, and add sterile glass beads if using beads to spread the mixture.

9. Pipet 20 µL from each tube onto the appropriate plate, and spread the mixture evenly across the plate. Do triplicates (3 each) of each tube if possible

10. Incubate at 37°C overnight. Position the plates so the agar side is facing up, and the lid is facing down.

11. Count the number of colonies on a light field or a dark background, such as a lab bench. Use the following equation to calculate your competent cell efficiency. If you've done triplicates of each sample, use the average cell colony count in the calculation.

11/06/2015

Goal :
Test transformation efficiency of our competent cells v2 and made glucose stock solution.

Procedure :

- Results of Transformation Efficiency of chemical competent cells v2

Plates n°	DNA quantity	Strain (-80°C or Azote)	LB + Antibiotic	UFC
1	**0,5 pg/µL**	Azote	**none**	bacterial lawn
2	5.0 pg/µL	Azote	**none**	bacterial lawn
3	0	Azote	**none**	bacterial lawn
4	**0,5 pg/µL**	-80°C	**none**	bacterial lawn
5	5.0 pg/µL	-80°C	**none**	bacterial lawn
6	0	-80°C	**none**	bacterial lawn
7	**0,5 pg/µL**	Azote	Chloramphenicol : 10 µg/ml	0
8	**0,5 pg/µL**	Azote	Chloramphenicol : 10 µg/ml	0
9	**0,5 pg/µL**	Azote	Chloramphenicol : 10 µg/ml	0
10	5.0 pg/µL	Azote	Chloramphenicol : 10 µg/ml	0
11	5.0 pg/µL	Azote	Chloramphenicol : 10 µg/ml	0
12	5.0 pg/µL	Azote	Chloramphenicol : 10 µg/ml	0
13	**0,5 pg/µL**	-80°C	Chloramphenicol : 10 µg/ml	0
14	**0,5 pg/µL**	-80°C	Chloramphenicol : 10 µg/ml	0
15	**0,5 pg/µL**	-80°C	Chloramphenicol : 10 µg/ml	0
16	5.0 pg/µL	-80°C	Chloramphenicol : 10 µg/ml	1
17	5.0 pg/µL	-80°C	Chloramphenicol : 10 µg/ml	0
18	5.0 pg/µL	-80°C	Chloramphenicol : 10 µg/ml	0

Results :
We obtained : 2,5 x 10^6 transformants/ µg of DNA on plate n°16.
Low transformation efficiency observed may have been caused if we regenerated cells in LB without glucose instead of SOC medium or LB+glc.
So we will prepare a glucose solution stock to optimized the regeneration step and hopefully obtain more transformants.

Remarks :
Petri dishes are too small, it is annoying. Moreover agar is full of little bubbles, it is very boring to distinguish colonies and bubbles.

- Preparation Glucose 2M stock (150mL)

- Dissolve 50,48 g glucose into 150 mL (final volume) ddH2O
- filter-sterilize into sterile 500 ml tube
- Store at 4°C

- Transformation Chemical cells (Azote) with pSB3C3, pSB3T5, (+pSB1C3 control)

Materials :

Resuspended DNA (Resuspend well in 10µl dH20, pipette up and down several times, let sit for a 5 minutes)
Competent cells (50µl per transformation)
LB liquid media (10 mL LB + 100µL glucose 2M)
Petri dishes with LB agar and appropriate antibiotic (2 per transformation)
Petri dishes with LB agar without antibiotic (1 per transformation)
10pg/µL RFP Control (pSB1C3 w/ BBa_J04450)

Protocol :

0. Resuspended well in 10µl dH20, pipette up and down several times, let sit for a 5 minutes
1. Spin down the DNA tubes to collect all of the DNA into the bottom of each tube prior to use. A quick spin of 20-30 seconds at 8,000-10,000 rpm will be sufficient. Note: There should be 50 µL of DNA in each tube sent in the Kit.
2. Thaw competent cells on ice. Label one 2.0mL microcentrifuge tube for each concentration and then pre-chill by placing the tubes on ice.
3. Pipet 1 µL of DNA into each microcentrifuge tube (pSB3C3, pSB3T5, pSB1C3). For each concentration, use a separate tube.
4. Pipet 50 µL of competent cells into each tube. Flick the tube gently with your finger to mix. Incubate on ice for 30 minutes. Pre-heat waterbath now to 42°C.
5. Heat-shock the cells by placing into the waterbath for 45sec. Be careful to keep the lids of the tubes above the water level, and keep the ice close by.
6. Immediately transfer the tubes back to ice, and incubate on ice for 5 minutes. This helps the cells recover.
7. Add 200 µL of LB+glucose (20mM final) media per tube, and incubate at 37°C for 1 hours.
8. Prepare the agar plates during this time: label them,
9. Label two petri dishes with LB agar and the appropriate antibiotic (ChL+Tet) with the part number, plasmid backbone, and antibiotic resistance.
10. Plate 20 µl and 200 µl of the transformation onto the dishes, and spread. This helps ensure that you will be able to pick out a single colony.
11. For the control, label two petri dishes with LB agar (ChL). Plate 20 µl and 200 µl of the transformation onto the dishes, and spread.
12. For control bacterial survey, plate 20µL on one LB agar without antibiotic by transformation
13. Incubate at 37°C overnight. Position the plates so the agar side is facing up, and the lid is facing down.

=> OBSERVATION on the 15/06/2015

12/06/2015

Goal :

Made LB agar plates and we have no colonies, so we will wait until monday .

Procedure :

- LB agar (X2) 500ml (= 20 Petri dishes 10cm)

LB agar	500 mL
Bactotryptone	5 g
Yeast Extract	2,5 g
NaCl	5 g
Agar	7,5g
(NOT DONE : Adjust pH to 7.0 with NaOH) and autoclave for 20 minutes	
+ Chloramphenicol (34 mg/ml in ethanol)	147 μL / 500 mL medium {10 μg/ml (final)}
+Tetracycline (12,5 mg/ml in 50% ethanol)	500 µL / 500 mL medium {12,5 µg/ml (final)}

Pouring the Plates

1. Make sure bench top has wiped down with bleach/EtOH.
2. Remove sterile Petri dishes from plastic bag
3. Pour a thin layer (5mm) of LB Agar (~10mL) into each plate being careful not to lift the cover off excessively (you should be able to just open up enough to pour).
4. Swirl plate in a circular motion to distribute agar on bottom completely.
5. Let each plate cool until it is solid (~20 minutes) then flip so as to avoid condensation on the agar.
6. Store plates in plastic bags in a cold room (4°C) with: name, date and contents

- M63 Medium

Component	Volume & Mass	Procedure
$(NH_4)_2SO_4$	10 g	1) Add the following reagents to a 2-liter flask 2) Adjust volume to 1 L 3) Once the ingredients are added, heat with stirring until the components are completely dissolved. 4) Adjust pH to 7.0 with Acid 5) Sterilize by autoclave
KH_2PO_4	68 g	
$FeSO_4.7H_2O$	2,5 **mg**	

15/06/2015

Goal :
Made antibiotic stock solution, counted colonies, selected positive clones and started a culture for miniprep.

Procedure :

- Ampicillin stock solution (50 mg ml-1)(40 ml)

1. Weigh out 2 g ampicillin (sodium salt) and dissolve in 35 ml of distilled water.
2. Make up volume to 40 ml with distilled water.
3. Sterilize by filtration.

<STORAGE> Aliquots of appropriate volume should be stored at -20 °C.
<USAGE> For culture plates, allow media to cool to 55 °C before adding ampicillin to a final concentration of 50 µg/ml.

- Kanamycin stock solution (50 mg ml-1)(40 ml)

1. Weigh out 2 g kanamycin sulfate and dissolve in 35 ml of distilled water.
2. Make up volume to 40 ml with distilled water.
3. Sterilize by filtration.

<STORAGE> Aliquots of appropriate volume can be stored at -20 °C.
<USAGE> For culture plates, allow media to cool to 55 °C before adding kanamycin to a final concentration of 50 µg/ml.

- Counting of Transformation Chemical cells (Azote) with pSB3C3, pSB3T5, (+pSB1C3 control)

Plasmid	DNA quantity	Spread Volume	Strain DH5-alpha	LB + Antibiotic	UFC
psB1C3	10 pg/µL	20 µL	Azote	none	bacterial lawn
psB3C5	**200-300pg/µL ??**	**20 µL**	**Azote**	**none**	**bacterial lawn**
psB3T5	200-300pg/µL ??	20 µL	Azote	none	bacterial lawn
psB1C3	10 pg/µL	20 µL	Azote	Chloramphenicol : 10 µg/ml	1 white colony
psB1C3	10 pg/µL	200µL	Azote	Chloramphenicol : 10 µg/ml	2 red colony
psB3C5	**200-300pg/µL ??**	**20 µL**	**Azote**	**Chloramphenicol : 10 µg/ml**	**0**
psB3C5	**200-300pg/µL ??**	**200µL**	**Azote**	**Chloramphenicol : 10 µg/ml**	**6 redd colony 6 white colony 3-4 small colony**
psB3T5	200-300pg/µL ??	20 µL	Azote	Tetracycline : 12,5 µg/ml	0
psB3T5	200-300pg/µL ??	200µL	Azote	Tetracycline : 12,5 µg/ml	1 red colony

- Reception of Nicolas' s request to iGEM HQ for yeast plasmid

Bba_J63005 ,Amp-R : orange cap
Bba_K319043, Chlor-R : green cap

- Select Clones of pSB3T5 and pSB3C5 by Picking Colonies Protocol

1. After taking the plates out of the 37ºC incubator place them upside down (i.e. the way they were in the incubator) on the bench top.

2. Using a pipetteman or a similar instrument, pipette 5 ml of LB media*** containing the correct concentration of antibiotic into sterile 25 ml or 50 ml tubes

(The number of tubes depends on how many you want to grow)
Tetracyclin : 12,5 µg/mL
Chloramphenicol : 10 µg/mL

3. In one hand take a sterile pipette tip on the end of a pipette, with the other hand pick up the upside down plate containing the bacteria from the ligation. Turn the plate over in your hand so that the bacteria are now facing upwards towards you and touch the tip of the pipette tip gently to a bacterial colony that is completely isolated from any other colony.

=> The Petri dishes are stocked in parafilm at 4°C.

4. Now place the same tip with the bacteria on it into one of the tubes containing LB media and move the tip around a bit to release some of the bacteria into the liquid. Some people simply eject the pipette tip into the media but if you do this you will need to recover it the next day.

5. Culture the tubes overnight in an incubated orbital shaker at 37ºC at 190-225 rpm.

*** Preparation of LB media :
Chloramphenicol : 5mL x 12 colonies + 5 (to be safe) = 65mL
Chloramphenicol (final) : 10 µg/mL
Chloramphenicol (stock) : 34 mg/mL

Tetracyclin : 5mL x 1 colony + 5 = 10 mL
Tetracyclin (final) : 12,5 µg/mL
Tetracyclin (stock) : 12,5 mg/mL

Vi = (Cf x Vf) / Ci

20µL of Chloramphenicol into 65 mL LB media
10µL of Tetracyclin into 10 mL LB media

=> 12 colonies for pSB3C5
=> 1 colony for pSB5T5

Next Morning :
For pSB3C5 : Colonies 1,2,3,4,5,6 and 11 = Red pellet / Colony 7,8,9,10 and 12 = No pellet
For pSB3T5 : Colony 1 = Red pellet

16/06/2015

Goal :

miniprep on positive colonies, preparation of yeast biobrick (ordered to iGEM by Nicolas) and transformation of them.

Procedure :

- LB agar 250ml (x2) = 10 Petri dishes 10cm

LB agar	250 mL
Bactotryptone	2,5 g
Yeast Extract	1,25 g
NaCl	2,5 g
Agar	4 g
(NOT DONE : Adjust pH to 7.0 with NaOH) and autoclave for 20 minutes	
Ampicillin stock solution (50 mg ml-1)(40 ml) + Kanamycin stock solution (50 mg ml-1)	250 µL / 250 mL medium {50 µg/ml. (final)} 250 µL / 250 mL medium {50 µg/ml. (final)}
Ampicillin stock solution (50 mg ml-1)(40 ml)	250 µL / 250 mL medium {50 µg/ml. (final)}

- Pour 20 Petri dishes.
- Store at 4°C.

- Preparation of Nicolas' Requested yeast plasmid

Bba_J63005 , Amp-R : orange cap
Bba_K319043 , Chlor-R : green cap

1. The agar will have a hole from when it was stabbed.
2. Dip an inoculating loop into the stab
3. Plate onto a petri dish of LB agar with Ampicillin or Chloramphenicol
4. Incubate the dish overnight at 37C (14-16hr)

- Storage of pSB1AK3 (plate 4, well 12B) and psB1C3+Bba_I14033 (plate3-13D)

- Resuspended well in 10µl dH20, pipette up and down several times, let sit for a 5 minutes
- Transfert into eppendorf tube
- Storage 4°C

- Transformation with pSB1AK3 (plaque 4, well 12B), pSB1C3+Bba_I14033 (pCAT) and pSB1C3-RFP (control)

Protocols : Heat-Shock Transformation of chemically competent bacteria

- Miniprep on selected colony for pSB3C5 and pSB3T5

Protocol : QIAprep Spin Miniprep Kit Using a Microcentrifuge on Select Clones pSB3T5 and pSB3C5

This protocol is designed for purification of up to 20 µg of high-copy plasmid DNA from 1–5 ml overnight cultures of E. coli in LB (Luria-Bertani) medium.

Note: All protocol steps should be carried out at room temperature.

=> Tube 1,2,3,4,5,6 and 10 for pSB3C5
=> Tube 1 for pSB3T5

Procedure :

0. Centrifuge culture 6min at 4400g.

1. Resuspend pelleted bacterial cells in 250 µl Buffer P1 and transfer to a microcentrifuge tube.

2. Add 250 µl Buffer P2 and gently invert the tube 4–6 times to mix.
- *Do not vortex*
- *Do not allow the lysis reaction to proceed for more than 5 min.*

3. Add 350 µl Buffer N3 and invert the tube IMMEDIATELY but GENTLY 4–6 times.

4. Centrifuge for 10 min at 13,000 rpm (~17,900 x g) in a table-top microcentrifuge.

5. Apply the supernatants from step 4 to the QIAprep spin column by decanting or pipetting.

6. Centrifuge for 30–60 s. Discard the flow-through.

7. Wash the QIAprep spin column by adding 0.5 ml Buffer PB and centrifuging for 30–60 s. Discard the flow-through.This step is required for low copy number plasmid.

8. Wash QIAprep spin column by adding 0.75 ml Buffer PE and centrifuging for 30–60 s.

9. Discard the flow-through, and centrifuge for an additional 1 min to remove residual wash buffer.

.
10. Place the QIAprep column in a clean 1.5 ml microcentrifuge tube.
To elute DNA,add 50 µl Buffer EB (10 mM Tris·Cl, pH 8.5) or water to the center of each QIAprep spin column, let stand for 1 min, and centrifuge for 1 min.

11. Storage at 4°C

17/06/2015

Goal :

Made a miniprep on positive colony, preparation of yeast biobrick (ordered to iGEM by Nicolas) and transformation of them.

Procedure :

- Miniprep verification by Restriction digestion (NotI) on agarose gel

8 Digestions (7 pSB3C5 + 1 pSB3T5)

1. Quickly vortex all ingredients before beginning.
2. Add the following in a micro-centrifuge tube:

- 5µL NEBuffer 3
- 3µL ADN (normally 1µg)
- 41µL dH2O
- 1µL NotI enzyme (NEB for tube 1,1' and Fermentas for tube 2,3,4,5,6,10)

3. Incubate reaction in a 37°C water bath for at least one hour.

2% Agarose gel : 1g agarose + 50 mL TBE
70mV, DNA ladder 100kb and 1 kB.

- Select Clones pSB3T5 and pSB3C5

Medium	Plasmid	CFU
LB	pSB1C3-BbaJ04450 (control)	Bacteria lawn
LB	pSB1AK3-BbaJ04450 (RFC)	Bacteria lawn
LB	pSB1C3-Bba_I14033 (pCat)	Bacteria lawn
LB + Chloro (10*µ*g/mL)	pSB1C3-BbaJ04450 (control 10pg/*µ*L)	200*µ*L : 0 colonies 20 *µ*L : 0 colonies
LB + AMP+ KAN (50 *µ*g/mL)	pSB1AK3-BbaJ04450 (RFC)	200*µ*L : 5 colonies 20 *µ*L : 0 colonies
LB + Chloro (10*µ*g/mL)	pSB1C3-Bba_I14033 (pCat)	200*µ*L : 9 colonies 20 *µ*L : 1 colony
LB + AMP (50 *µ*g/mL)	BbaJ3010-BbaJ3005	Stripes
LB + Chloro (10*µ*g/mL)		Stripes

- Picking Colonies Protocol

1. After taking the plates out of the 37ºC incubator place them upside down (i.e. the way they were in the incubator) on the bench top.

2. Using a pipette boy or similar instrument, pipette 5 ml of LB media*** (containing the correct concentration of antibiotic into sterile 25 ml or 50 ml tubes

(The number of tubes depends on how many you want to grow)

Ampicillin+Kanamycin : 12,5 µg/mL
Chloramphenicol : 10 µg/mL

3. In one hand take a sterile pipette tip on the end of a pipette, with the other hand pick up the upside down plate containing the bacteria from the ligation. Turn the plate over in your hand so that the bacteria are now facing upwards towards you and touch the tip of the pipette tip gently to a bacterial colony that is completely isolated from any other colony.

=> The Petri dishes are stock in parafilm at 4°C.

4. Now place the same tip with bacteria on it into one of the tubes containing LB media and move the tip around a bit to release some of the bacteria into the liquid. Some people simply eject the pipette tip into the media but if you do this you will need to recover it the next day.

5. Culture the tubes overnight in an incubated orbital shaker at 37ºC at 190-225 rpm.

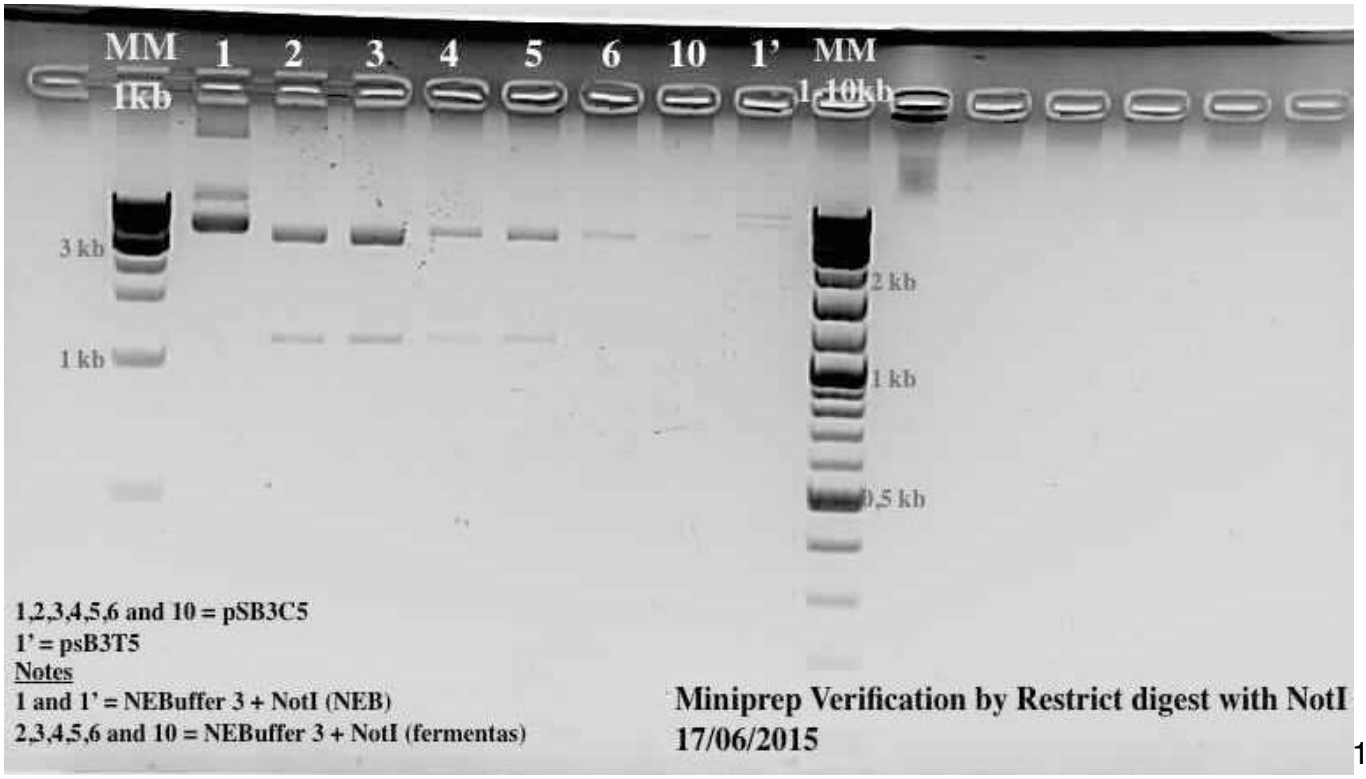
***** Preparation of LB media :**
Chloramphenicol : 5mL x 12 colony = 70mL
Chloramphenicol (final) : 10 µg/mL
Chloramphenicol (stock) : 34 mg/mL

Ampicillin+Kanamycin : 5mL x 2 colony = 10 mL
Ampicillin+Kanamycin (final) : 50 µg/mL
Ampicillin+Kanamycin (stock) : 50 mg/mL

Vi = (Cf x Vf) / Ci

20µL of Chloramphenicol into 70 mL LB media
10µL of Ampicillin + 10µL of Kanamycin into 10 mL LB media

=> 10 colonies for pSB1C3-Bba_I14033 (pCat- + 2 colony for Bba_K31943 (iGEM request)
=> 4 colonies for pSB1AK3 (Nicolas backbone)



pSB3C5-Bba_J04450 = 3807bp => NotI (1079 and 3793)
pSB3T5-Bba_J04450 = 4321bp => NotI (1079 and 4307)
Bba_J04450 = 1069bp

18/06/2015

- Miniprep on selected colony for pSB1AK3, pSB3T5

Protocol: QIAprep Spin Miniprep Kit Using a Microcentrifuge on Select Clones pSB3T5 and pSB3C5

This protocol is designed for purification of up to 20 µg of high-copy plasmid DNA from 1–5 ml overnight cultures of E. coli in LB (Luria-Bertani) medium.

Notes : All protocol steps should be carried out at room temperature. Storage at 4°C

=> Tube 1,2,3,4,5,6,7,8,9 and 10 for pSB1AK3
=> Tube 1 for pSB3T5

- PCR Verification of minipreps of psB3T5, pSB3C5, psB1AK3, pCat (Bba_I14033) and Bba_K319043 (iGEM Nicolas)

=>Primers reception (IDT) : stock solution 100µM (storage -20°C)
aliquot 10µM (10µL primers + 90µL H_2O)

The following table shows all of the PCR's that have been done.

Mix Phusion
MIX pour 1 échantillon

5X Phusion GC Buffer	4	µL
dNTP Mix	0,4	µL
amorce L 10 µM	1	µL
amorce R 10µM	1	µL
PhUsion DNApol	0,2	µL
eau	11,4	µL
ADN	2	µL
	20	

	95°C	5'
	95°C	30"
	50°C	1'
	72°C	1'
	72°C	5'

30 fois

MIX	26	échantillons
5X Phusion GC Buffer	104,00	µL
dNTP Mix	10,4	µL
VF2 (10µM)	26,00	µL
VR (10µM)	26,00	µL
PhUsion DNApol	5,2	µL
eau	296,40	µL
Vf=	468,00	µL

1) pSB3C5-Bba_J04450	1) pSB1AK3	1) pCat (Bba_I14033)	**H20**
2) pSB3C5-Bba_J04450	2) pSB1AK3	2) pCat (Bba_I14033)	
3) pSB3C5-Bba_J04450	3) pSB1AK3	3) pCat (Bba_I14033)	
4) pSB3C5-Bba_J04450	4) pSB1AK3	4) pCat (Bba_I14033)	
5) pSB3C5-Bba_J04450	1) Bba_K319043	5) pCat (Bba_I14033)	
6) pSB3C5-Bba_J04450	2) Bba_K319043	6) pCat (Bba_I14033)	
10) pSB3C5-Bba_J04450	*9) pCat (Bba_I14033)*	7) pCat (Bba_I14033)	
1') pSB3T5-Bba_J04450	*10) pCat (Bba_I14033)*	8) pCat (Bba_I14033)	

19/06/2015

- Miniprep verification by PCR product on gel agarose 1%

Results : No analysis because too much DNA

- Miniprep quantification by nanodrop

1 / pSB3C5-BBa_J04450 : 501,8ng/µL
2 / pSB3C5-BBa_J04450 : 301,9 ng/µL
3 / pSB3C5-BBa_J04450 : 389,0 ng/µL
4 / pSB3C5-BBa_J04450 : 124,0 ng/µL
5 / pSB3C5-BBa_J04450 : 204,2 ng/µL
6 / pSB3C5-BBa_J04450 : 115,2 ng/µL
10 / pSB3C5-BBa_J04450 : 121,8 ng/µL

1' / pSB3T5-BBa_J04450 : 65 ng/µL

1 / pSB1AK3-BBa_J04450 : 122,6 ng/µL
2 / pSB1AK3-BBa_J04450 : 123,8 ng/µL
3 / pSB1AK3-BBa_J04450 : 136,3 ng/µL
4 / pSB1AK3-BBa_J04450 : 128 ng/µL

1 / BBa_K319043 : 253,7 ng/µL
2 / BBa_K319043 : 250,7 ng/µL

1 / pSB1C3-BBa_I14033 (pCat) : 47, 4 g/µL
2 / pSB1C3-BBa_I14033 (pCat) : 64,6 ng/µL
3 / pSB1C3-BBa_I14033 (pCat) : 57,8 ng/µL
4 / pSB1C3-BBa_I14033 (pCat) : 50,7 ng/µL
5 / pSB1C3-BBa_I14033 (pCat) : 48,4 ng/µL
6 / pSB1C3-BBa_I14033 (pCat) : 64,7 ng/µL
7 / pSB1C3-BBa_I14033 (pCat) : 61,7ng/µL
8 / pSB1C3-BBa_I14033 (pCat) : 69,0 ng/µL
9 / pSB1C3-BBa_I14033 (pCat) : 67,1 ng/µL
10 / pSB1C3-BBa_I14033 (pCat) : 70,8 ng/µL

- PCR Verification miniprep psB3T5, pSB3C5, psB1AK3, pCat (Bba_I14033) and Bba_K319043 (iGEM Nicolas) with 1ng DNA

=>Primers reception (IDT) : stock solution 100µM (storage -20°C)
aliquot 10µM (10µL primers + 90µL H2O)

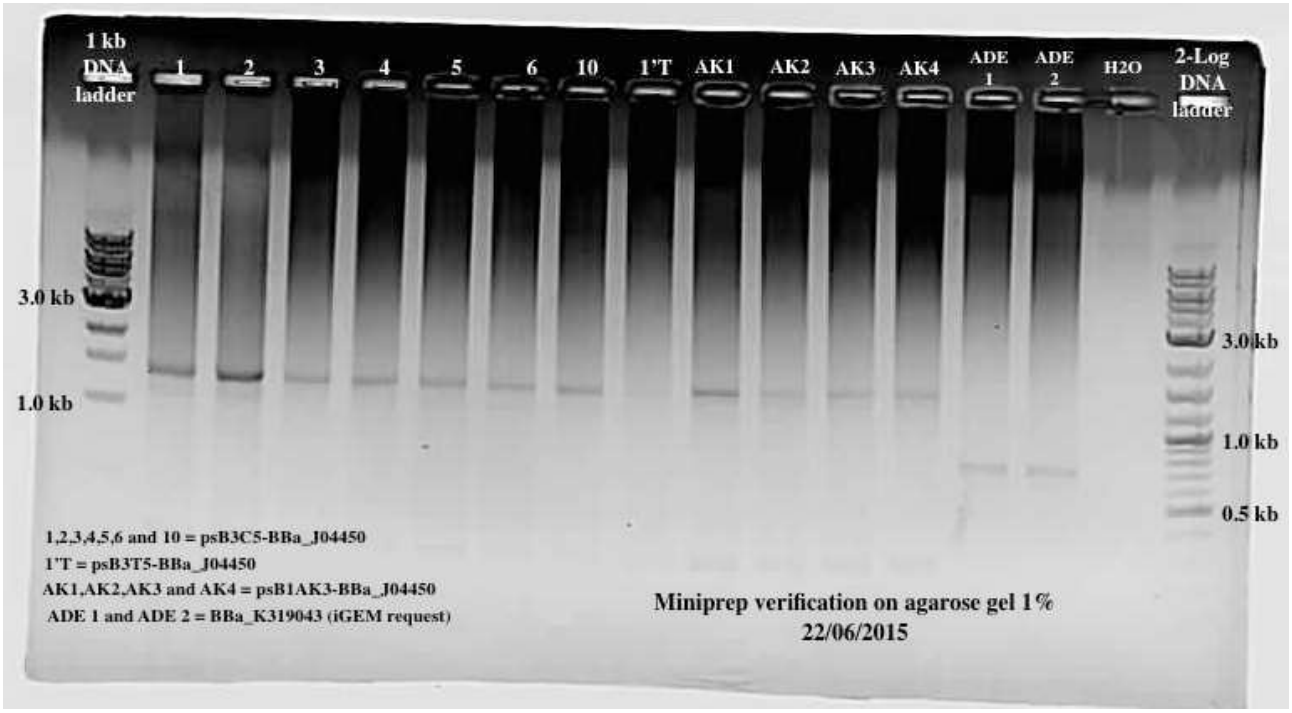
Procedure : Same protocol as on 18/06/2015 (page 19)

22/06/2015

- Miniprep verification by PCR product (19/06/2015) on gel agarose 1% and 2%

- Agarose gel 1% (0,5g agarose in 50mL TBE 0,5X), 10µL depot, DNA ladder 1kb and 2-log (1µL)

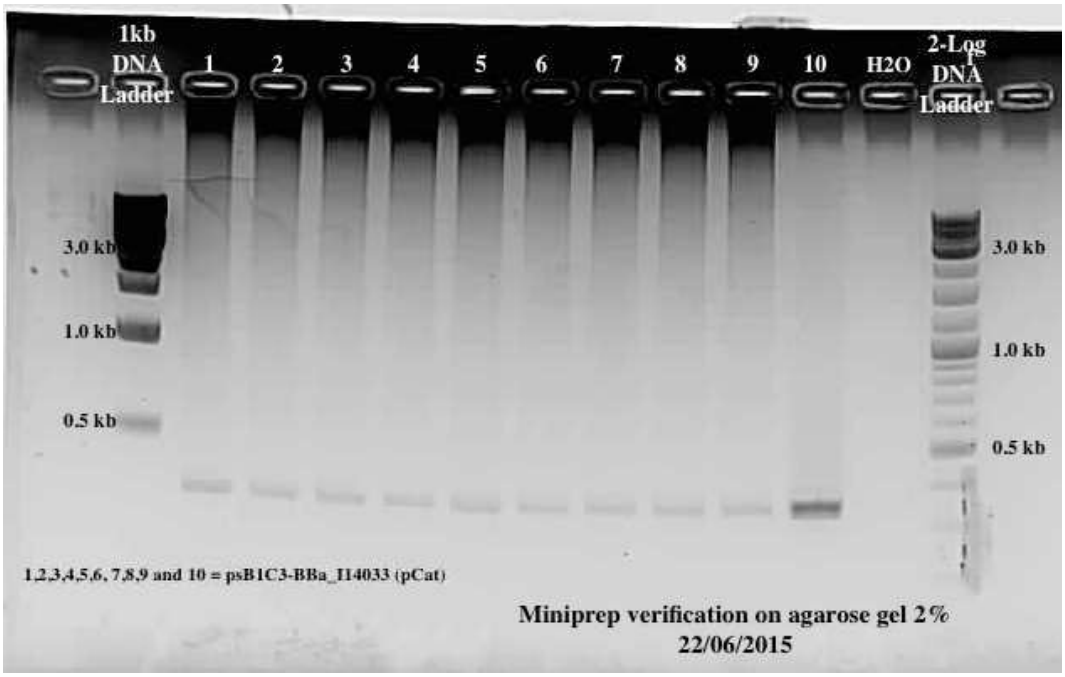
- pSB3C5-BBa_J04450 : amplicon = 1329 bp
- pSB3T5-BBa_J04450 : amplicon = 1335 bp
- pSB1AK3-BBa_J04450 : amplicon = 1363 bp
- BBa_K319043 : amplicon = 2821 bp **????**



- Agarose gel 2%(1g agarose in 50mL TBE 0,5X, 10µL depot, DNA ladder 1kb and 2-log (1µL)

- pSB1C3-BBa_I14033 (pCat) :amplicon = 332 bp

- SOB Medium : 500 mL



Final [c]	Component	Volume & Mass
2 %	Bactotryptone	10 g
0,5 %	Yeast Extract	2,5 g
10 mM	NaCl	1 ml (5 M stock)
2,5 mM	KCl	0,09 g
10 mM	$MgCl_2$	2,5 ml (2 M stock)
10 mM	MgSO4	10 mL (0,5 M)
-	H_20	QS 500 mL

pH media to 7 with NaOH, then filtration.

- Resuspending BBa_J45993 (162 bp) IDT

1. Centrifuge the tube for 5 sec at a minimum of 3000 x g to ensure the material is in the bottom of the tube
2. Add EB Buffer (Elution Buffer Miniprep) to reach a final concentration of 10ng/µL => 250 ng DNA + 25 µL EB buffer
3. Vortex briefly
4. Incubate at 50°C for 20min
5. Breifly vortex and centrifuge
6. Store at 4°C

- Test of Transformation Efficiency with Kit iGEM (again)

Protocol

1. Spin down the DNA tubes from the Transformation Efficiency Kit to collect all of the DNA into the bottom of each tube prior to use. A quick spin of 20-30 seconds at 8,000-10,000 rpm will be sufficient. We test 50 pg/µL and 20 pg/µL DNA concentration.

2. Thaw competent cells on ice. Label one 2.0mL microcentrifuge tube for each concentration and then pre-chill by placing the tubes on ice.

3. Pipet 1 µL of DNA into each microcentrifuge tube. For each concentration, use a separate tube.

4. Pipet 50 µL of competent cells into each tube (2 tubes for Azote and 2 tubes for -80°C freeze conditions). Flick the tube gently with your finger to mix. Incubate on ice for 30 minutes. Pre-heat waterbath now to 42°C.

5. Heat-shock the cells by placing into the waterbath for 45 sec. Be careful to keep the lids of the tubes above the water level, and keep the ice close by.

6. Immediately transfer the tubes back to ice, and incubate on ice for 5 minutes. This helps the cells recover.

7. Add 200 µL of LB media per tube, and incubate at 37°C for 1 hours.

8. Prepare the agar plates during this time: label them, and add sterile glass beads if using beads to spread the mixture.

9. Pipet 20 µL and 200 µL from each tube onto the appropriate plate, and spread the mixture evenly across the plate.

10. Incubate at 37°C overnight. Position the plates so the agar side is facing up, and the lid is facing down.

- Making DMSO Competent Cells « AGAIN »

Day One : Grow 6 ml overnight culture of favorite strain of E. coli in 2XTY at 37°C and 180 rpm (preheat medium at 37°C before inoculation)

- Dilution BBa_J45993 (162 bp) IDT.

We have 25 µL at 10 ng/µL.
We want 100µL at 0,05 ng/µL.
So we take 0,5 µL to stock solution (10 ng/µL) in 99,5 µL.

- PCR amplification of BBa_J4993 (162 bp)

- Forward primer : VF2
- Reverse primer : VR
- 2 tubes with 1µL of BBa_J4993 (0,05 ng/µL)
- 1 tube with 1µL dH2O

1 Cycle :
5min at 95°C

Component	20 µL reaction
ddH20	12,4 µL
5X Phusion GC Buffer	4 µL
10 mM dNTPs mix	0,4 µL
10 µM Forward Primer	1 µL
10 µM Reverse Primer	1 µL
Phusion DNApol	0,2 µL
DNA	1 µL
TOTAL	20

Mix	3 reactions
ddH20	37,2 µL
5X Phusion GC Buffer	12 µL
10 mM dNTPs mix	1,2 µL
10 µM Forward Primer	3 µL
10 µM Reverse Primer	3 µL
Phusion DNApol	0,6 µL
TOTAL	57

30 Cycles :
30sec at 92°C
30sec at 50°C
4sec at 72°C

1 Cycle :
5min at 72°C

23/06/2015

- Transformation Efficiency Kit iGEM « AGAIN »

11. Count the number of colonies on a light field or a dark background, such as a lab bench. Use the following equation to calculate your competent cell efficiency. If you've done triplicates of each sample, use the average cell colony count in the calculation.

Azote Freeze Bacteria :
pSB1C3-BBa_J04450 :
- 20pg/µL => 20µL = 0 colonies
  200µL = 3 colonies
- 50pg/µL => 20µL = 0 colonies
  200µL = 3 colonies

-80°C Freeze Bacteria :
pSB1C3-BBa_J04450 :
- 20pg/µL => 20µL = 0 colonies
  200µL = 2 colonies
- 50pg/µL => 20µL = 0 colonies
  200µL = 12 colonies

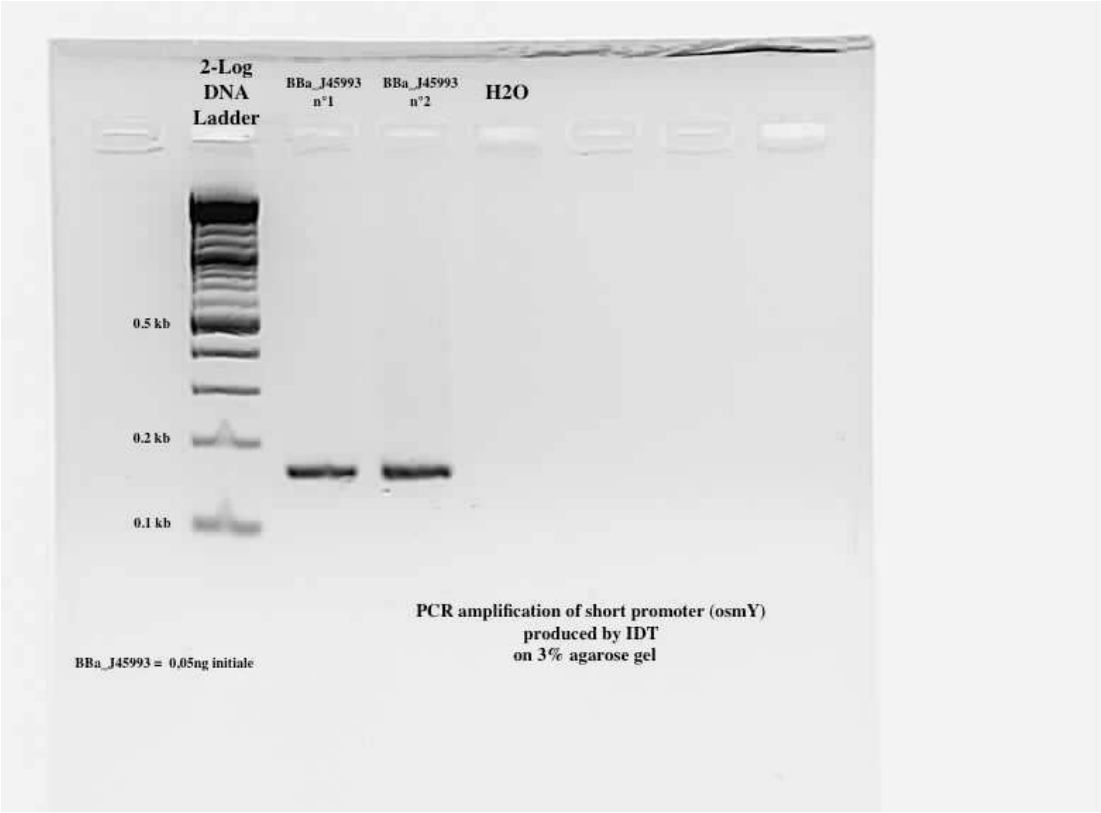
Calculation :
For 50pg/µL and 200µL plate :
{ 12 x (250/200) } / 50 = 0,3 transformants per pg of DNA => so 300 000 transformants per µg of DNA.

Conclusion :

!!!! Our bacteria are NOT competent since the efficacy should be much higher (around 10^8) !!!!!

- Verification of PCR amplification of BBa_J45993 (IDT) on agarose gel 3%

- 1g agarose + 30 mL TAE 0,5X
- 5µL of PCR reaction by wells (x2 because during the migration of the first gel DNA escaped from the gel to conquer the world !!!!) + 1µL Blue Loader
- 3µL 2-Log DNA ladder



- Making DMSO Competent Cells « AGAIN »

Day Two :

1) Keep 5mL of SOB for initial OD.
2) Inoculate 500 mL SOB with 6 ml overnight culture.
3) Grow culture at 180 rpm at 18°C (this temperature is really important as we see a 10-fold decrease in competency when we grow them at room temperature).

- SOB Medium : 500 mL

Final [c]	Component	Volume & Mass
2 %	Bactotryptone	10 g
0,5 %	Yeast Extract	2,5 g
10 mM	NaCl	1 ml (5 M stock)
2,5 mM	KCl	0,09 g
10 mM	$MgCl_2$	2,5 ml (2 M stock)
10 mM	MgSO4	10 mL (0,5 M)
-	H_20	qsp 500 mL

pH media to 7 with NaOH, then filtration.

- Preparation of Glycine/NaOH buffer ph 9.5

- Measure 15 g of Glycine (75,07 MW)
- Adjust at 200mL
- Adjust pH to 9.5 with NaOH 5N
- Autoclave

24/06/2015

- Making DMSO Competent Cells « AGAIN »

DAY THREE:

1) Grow cells until A_{600} 0.5-0.7.

Blank = SOB

A600 = 0,488

Subsequent steps should be carried out in the cold room on ice:

1) Dispatch the 500mL culture into 10 tubes of 50mL.

2) Put flask on ice for 10 minutes

3) Spin cells down at 2500xg (3350 RPM) for 10min at 4°C

4) Pour off supernatant

5) Resuspend gently first in 5 mL TB, then add remaining 11 ml (Final 16mL)

6) Leave on ice for 5 minutes

7) Spin down again at 2500xg for 10 minutes at 4°C

8) Resuspend cells in 2 ml TB

9) Pool all tubes in only one (Final 20mL)

10) Add 1,5 ml of DMSO dropwise while gently shaking [final DMSO concentration is 7%].

11) Aliquot in 100 µl aliquots (you will need about 450 pre-chilled 0.5 ml tubes).

12) Flash freeze in liquid nitrogen. NOT DONE

13) Directly store at -80°C .

- Resuspending BBa_J45992 (304 bp), crdA (1563 bp), crdS (2070 bp) and crdC (1371 bp) IDT

7. Centrifuge the tube for 5 sec at a minimum of 3000 x g to ensure the material is in the bottom of the tube
8. Add EB Buffer (Elution Buffer Miniprep XXX) to reach a final concentration of 10ng/µL =>
   BBa_J45992 = 500 ng DNA + 50 µL EB buffer
   crdA = 1000 ng DNA + 100 µL EB buffer
   crdS = 4 µg DNA + 400 µL EB buffer
   crdC = 1000 ng DNA + 100 µL EB buffer

9. Vortex briefly
10. Incubate at 50°C for 20min
11. Briefly vortex and centrifuge
12. Store at 4°C

- Dilution BBa_J45992, crdA, crdS and crdC IDT.

We have a concentration of 10 ng/µL of DNA in each tube.
We want :
For BBa_J45992 : 100µL to 0,05 ng/µL.
For crdA : 100µL to 0,5 ng/µL.
For crdS, 100µL to 1 ng/µL.
For crdC, 100µL to 0,5 ng/µL.

For BBa_J45992, we take 0,5 µL of stock solution (10 ng/µL) in 99,5 µL.
For crdA, we take 5 µL of stock solution (10 ng/µL) in 95 µL.
For crdS, we take 10 µL of stock solution (10 ng/µL) in 90 µL.
For crdC, we take 5 µL of stock solution (10 ng/µL) in 95µL.

These dilutions are stored at 4°C.

- PCR amplification of BBa_J45992 (304 bp), crdA (1563 bp), crdS (2070 bp) and crdC (1371 bp) IDT

- Forward primer : VF2
- Reverse primer : VR
- 2 tubes of each DNA concentration with 1µL of For BBa_J45992 (0,05 ng/µL), crdA (0,5 ng/µL), crdS (1 ng/µL), crdC (0,5 ng/µL)
- 1 tube with 1µL dH2O

1 Cycle :
5min at 95°C

30 Cycles :
30sec at 92°C
45sec at 50°C
2min 30sec at 72°C

1 Cycle :
5min at 72°C

Component	20 µL reaction
ddH20	12,4 µL
5X Phusion GC Buffer	4 µL
10 mM dNTPs mix	0,4 µL
10 µM Forward Primer	1 µL
10 µM Reverse Primer	1 µL
Phusion DNApol	0,2 µL
DNA	1 µL
TOTAL	20

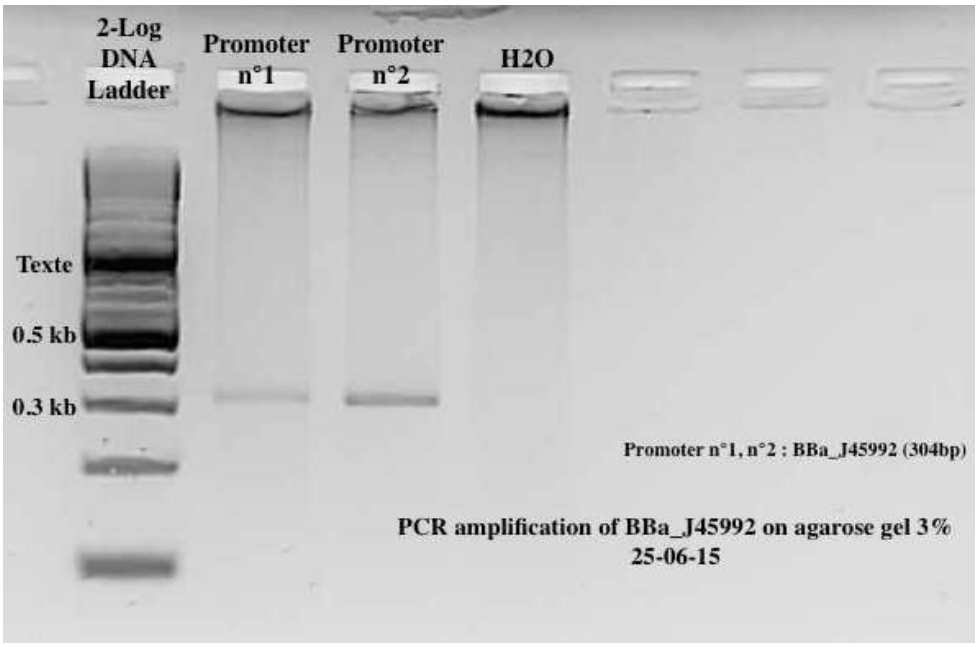
Mix	10 reactions
ddH20	124 µL
5X Phusion GC Buffer	40 µL
10 mM dNTPs mix	4 µL
10 µM Forward Primer	10 µL
10 µM Reverse Primer	10 µL
Phusion DNApol	2 µL
TOTAL	190

- **Transformation Efficiency Kit iGEM « On new DMSO competent cells »**

=> Same protocol as page 22.
We test with 50pg and 20pg on LB + Chloramphenicol (20 and 200µL)

25/06/2015

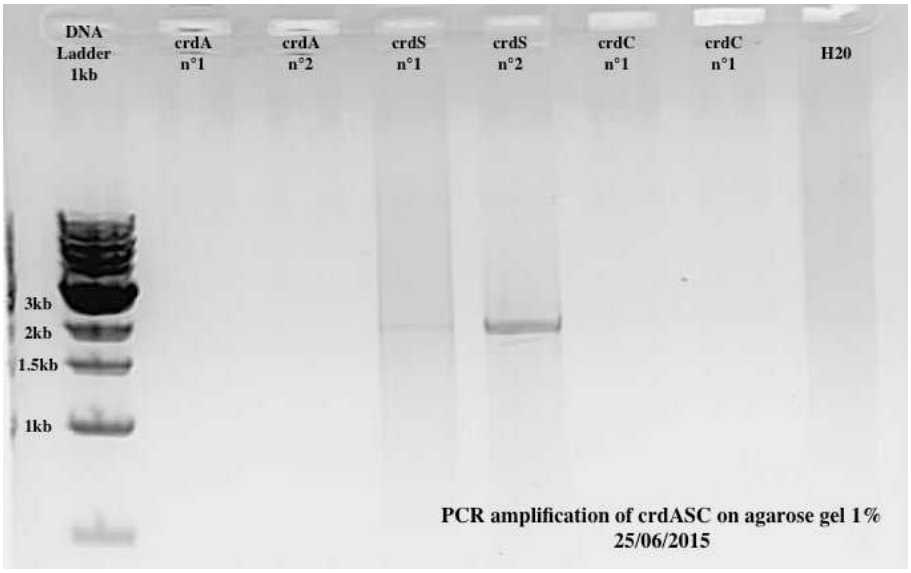
- Verification of PCR amplification of BBa_J45992 (IDT) on agarose gel 3%



(1g agarose + 30 mL TAE 0,5X)
- 5µL of PCR reaction by wells + 1µL Blue Loader
- 2µL DNA ladder 1kb

BBa_J45992 = OsmY promoter = 304 bp

- Verification of PCR amplification of crdASC on agarose gel 1%



(0,3g agarose + 30 mL TAE 0,5X)
- 5µL of PCR reaction by wells + 1µL Blue Loader
- 1µL 2-Log DNA ladder
- crdA (1563 bp), crdS (2070 bp) and crdC (1371 bp)

Results : Problem so we restart an other PCR on crdASC.

- PCR amplification of crdA (1563 bp), crdS (2070 bp) and crdC (1371 bp) IDT

- Forward primer : VF2
- Reverse primer : VR
- 2 tubes of each DNA concentration with 1µL of crdS (1 ng/µL),
- 2 µL of crdA (0,5 ng/µL) and crdC (0,5 ng/µL)

- 1 tube with 1µL dH2O

Component	20 µL reaction
ddH20	12,4 µL
5X Phusion GC Buffer	4 µL
10 mM dNTPs mix	0,4 µL
10 µM Forward Primer	1 µL
10 µM Reverse Primer	1 µL
Phusion DNApol	0,2 µL
DNA	1 µL
TOTAL	20

Mix	8 reactions
ddH20	99,2 µL
5X Phusion GC Buffer	32 µL
10 mM dNTPs mix	3,2 µL
10 µM Forward Primer	8 µL
10 µM Reverse Primer	8 µL
Phusion DNApol	1,6 µL
TOTAL	152

5min	95°C	
30sec	92°C	X 30
30sec	50°C	
2min 45sec	72°C	
5min	72°C	

- Transformation Efficiency Kit iGEM « On new DMSO competent cells »

Count the number of colonies on a light field or a dark background, such as a lab bench. Use the following equation to calculate your competent cell efficiency. If you've done triplicates of each sample, use the average cell colony count in the calculation.

- For 50 pg DNA :
=> 20µL : 72 colonies
=> 200µL : 58 colonies on 1/8 of the Petri dish = 464

- For 20 pg DNA :
=> 20µL : 22 colonies
=> 200µL : 115 colonies on 1/4 of the Petri dish = 460

Calculation :
({ (72 + 464) x (250/220) } / 50) x10^6 = 1,2 x 10^7 transformants / µg d'ADN
({ (22 + 460) x (250/220) } / 20) x10^6 = 2,7 x 10^7 transformants / µg d'ADN

Average = 1,9 x 10^7 transformants / µg d'ADN

WE HAVE DMSO COMPETENT CELLS NOW => LETS GO PARTY BEGINS !!!!!

- NaOH 10 N, 5N, 1N and 0,5 N solution stock

=> NaOH 10 N stock solution 100g of NaOH powders in 250 mL

- For 6N solution : 31,25 mL of 10 N stock solution + 18,75 mL H2O (Final 50 mL)
- For 1N solution : 5 mL of 10 N stock solution + 45 mL H2O (Final 50 mL)
- For 0,5N solution : 25 mL of 10 N stock solution + 75 mL H2O (Final 500 mL)

- LB agar and LB agar + Chloramphenicol

- 6g agar in 500 mL LB liquid + (after heating) 150 µL Chloramphenicol (34mg/mL stock)
- 3 g agar in 500 mL LB liquid
- Heat in the microwave 30 min
- Plate on Petri dish

Storage at 4°C : 9 LB agar and 19 LB agar + Chloro (10 µg/mL)

26/06/2015

- Prepare DYE MIX :

16,7 mL : 40 volumes of 0.1% aniline blue in water
8,75 mL : 21 volumes of 1 N HCl
24,6 mL : 59 volumes of 1 M glycine/NaOH buffer pH 9.5

- Resuspend of pSB1C3-BBa_B0015 of Plate Kit iGEM (Double Terminator, 129 pb, Well 3F, Plate 3)

75µL whereas 10 µL H20
Storage 4°C

- Transformation of pSB1C3-BBa_B0015

- Transformation with 5µL of pSB1C3-BBa_B0015
- 50 µL of chimio-competent cells
- Spread 20 µL, 20 µL, 10 µL and 100 µL on agar LB+Chlororamphenicol (10 µg.mL-1)

29/06/2015

- Pick select colony

- recovery of petri dishes containing BBa_B0015 in LB medium + chloramphenicol
- transplant of one colony in 5mL of LB liquid medium + chloramphenicol (X6)
=> 35 mL of medium prepared with 10,2µL of chloramphenicol at 10µg.mL^{-1}
=> put at 37°C, 180 rpm during 24h

- Quantification of Curdlan

Standard Range :
Curdlan stock solution : 20 mg in 10 mL(2mg/mL)
Dilute to obtain 20, 15, 10, 5 µg/mL sample solution.

Diluted sample 10-50-fold with1 N NaOH to a final volume of 300 µL in a 1.5 mL microcentrifuge tube.

1) Add 30 µL of 6 N NaOH
2) Incubated at 80 °C for 30 min.
3) The tube was immediately put on an ice bath.
4) Add 630 µL DYE MIX* into the tube and mix
5) Incubated at 50 °C for 30 min
6) The unbound fluorescent dye was decolorized at room temperature for 30 min

- BioBrick Assembly Kit of NEB

Following protocol kit.

- **Upstream part digest with EcoRI and SpeI**

PCR of BBa_J45992 (123,8 ng/µL) => 4 µL

- **Downstream part digest with XbaI and PstI**

PCR of CrdS PCR 2 / tube 1 (200 ng/µL) => 2,5 µL

- **Destination Plasmid digest with EcoRI and PstI**

pSB3C5-BBa_J04450 (501,8 ng/µL) => 1,5 µL
pSB1C3-BBa_I14033 (50ng/µL) => 10 µL

- Nanodrop Dosage of PCR product

Blanck = H20 sample PCR

1/ CrdS PCR n°1 (24/06/2015) : 113,9 ng/µL
2/ CrdS PCR n°1 (24/06/2015) : 121,7 ng/µL

1/ CrdS PCR n°2 (25/06/2015): 199,3 ng/µL
2/ CrdS PCR n°2 (25/06/2015): 251,6 ng/µL

1/ BBa_J45993 PCR (23/06/2015): 38,7 ng/µL
2/ BBa_J45993 PCR (23/06/2015): 168,2 ng/µL

1/ BBa_J45992 PCR (24/06/2015): 123,8 ng/µL
2/ BBa_J45992 PCR (24/06/2015): NS ng/µL

NOT REALLY TRUSTWORTHY because we have not purifed our PCR Product !!!!

30/06/2015

- Transformation of pSB1C3-BBa_J45992-CrdS + pSB3C5-BBa_J45992-CrdS

- Transformation with 2µL of pSB1C3-BBa_J45992-CrdS and pSB3C5-BBa_J45992-CrdS
- 50 µL of chimio-competent cells

- Spread 20 µL, 20 µL and 200 µL on agar LB+Chlororamphenicol (10 µg.mL-1)

- Miniprep of Pick selected colony (BBa_B0015)

Like 18/06/2015

- Quantification of Miniprep BBa_B0015 on nanodrop

Tube 1 : 60,3 ng/µL
Tube 2 : 137,0 ng/µL
Tube 3 : 52,1 ng/µL
Tube 4 : 50, 3 ng/µL
Tube 5 : 49,7 ng/µL
Tube 6 : 54,6 ng/µL

- PCR verification of Miniprep of BBa_B0015 (2X-Terminator) :

- Forward primer : VF2 (10µM)
- Reverse primer : VR (10µM)

1µL of BBa_B0015 (1 ng/µL) for each tube (6),
- 1 tube with 1µL dH2O

Component	20 µL reaction
ddH20	12,4 µL
5X Phusion GC Buffer	4 µL
10 mM dNTPs mix	0,4 µL
10 µM Forward Primer	1 µL
10 µM Reverse Primer	1 µL
Phusion DNApol	0,2 µL
DNA	1 µL
TOTAL	20

Mix	8 reactions
ddH20	99,2 µL
5X Phusion GC Buffer	32 µL
10 mM dNTPs mix	3,2 µL
10 µM Forward Primer	8 µL
10 µM Reverse Primer	8 µL
Phusion DNApol	1,6 µL
TOTAL	152

5min	95°C	
30sec	92°C	X 30
30sec	50°C	
4 sec	72°C	
5min	72°C	

- YPD Medium preparation

Composition	Mass for 500 mL liquid	Mass for 250 mL solid
Yeast extract 1%	5 g	2,5 g
Bacto-peptone 2%	10 g	5 g
Agar	-	10 g
H_2O	QS 500 mL	QS 250 mL

Autoclavage :
30 min at 110°C to conserve glucose
Plate 10 dishes in a laminar flow hood